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EDITORIAL REVIEW

Heat shock proteins and innate immunity

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Heat shock proteins (hsp) have attracted considerable attention from immunologists over the last 20 years, and their interest has evolved in three distinct phases. Initially hsp were investigated primarily as antigens, particularly when it was found that they were rather common targets of both the humoral and T cell-mediated responses to intracellular pathogens like mycobacteria. Their recognition by T cells in models of autoimmune disease, particularly arthritis and diabetes, gave rise to much speculation that immune responses initially directed against hsp from pathogens might cross-react with self antigens including hsp themselves [1,2]. Since hsp are often up-regulated at sites of inflammation this would provide opportunities for persistent stimulation of cross-reactive hsp-specific T cells [3]. Such speculation continues, although more recent evidence points to anti-inflammatory properties of T cells which recognize self hsp rather than their ability to induce autoimmune disease [4].

A second involvement of hsp in T cell-mediated immunity was demonstrated by the pioneering studies of Srivastava and colleagues, who showed that the chaperone function of many hsp (their ability to bind to and protect other polypeptides) allows them to deliver tumour antigens very effectively to antigen presenting cells [5,6]. This delivery appears to be receptor mediated, and hsp receptors are now being characterized, such as CD91 which binds several different hsp including two members of the hsp90 family and hsp70 [7]. In addition, when fusion proteins are created which contain antigen and part of the sequence of hsp70, the gain in immunogenicity is very marked, especially for the induction of responses by CD8+ T cells [8,9]. This function is analogous to that reported for antigens coupled to fragments of C3 which are potent inducers of antibody responses [10]. Thus both C3 and hsp might be regarded as physiological adjuvants, and serve as links between the innate and the acquired immune systems.

This brings us to the third immunological role of hsp, namely their ability to stimulate cells of the innate immune system, particularly antigen presenting cells, though interactions with other myeloid cells and endothelial cells have also been described. The initial observations were that hsp, usually tested as recombinant proteins, could elicit production of cytokines such as IL-1 or TNFα from monocyte/macrophage cells and cell lines [11–16]. Another such paper by Ueki *et al*. [17] appears in this issue of CEI and documents cytokine production by macrophages in response to human hsp60; interestingly the investigators' initial interest seems to have been in hsp60 from *Actinobacillus actinomycetemcomitans,* a bacterium implicated in periodontal disease, but this did not induce cytokine production. Their initial idea that the bacterial hsp might mediate periodontal disease was modified therefore to suggest that human hsp60 released in response to infection would contribute to bone loss. In view of the accumulating literature on intrinsic stimulatory properties of hsp, it is timely to review current evidence on this novel feature of hsp.

A brief summary of the effects which have been measured and the hsp which have been implicated is shown in Table 1; this is not exhaustive and not all hsp have been tested in every system. The table also lists hsp by family but this conceals species differences reported by several workers; as noted already Ueki *et al*. [17] described a response mediated by human hsp60 but not hsp60 from two oral pathogens. Other workers have reported no effect of mycobacterial hsp60 in a bone resportion assay, but activity from *E. coli* hsp60 (GroEL). These results are surprising because in other studies mycobacterial hsp60 has been reported to be effective in producing effects on endothelial cells, and macrophages. This goes against the simple assumption that members of each hsp family interact with the same receptor on target cells, with variation in the effects observed reflecting the properties of the target cell. There are certainly difficulties in postulating receptors which can make relatively fine distinctions between members of the same hsp family from different species, given the conserved amino acid sequence and structure which is characterisic of hsp.

All workers in this field have had to contend with the suggestion that their hsp preparations, being derived from recombinant proteins, contain LPS (and other bacterial products), and that it is these which are responsible for cytokine induction or activation of myeloid and endothelial cells. Indeed, it is the case that most of the effects documented to occur with hsp can be duplicated by LPS. In addition the amounts of hsp required are usually in the 1–10 μg/ml range whereas LPS produces similar effects at nanogram/ml concentrations. Thus there is considerable potential for artefact if careful controls are not included in experiments. The standard defence mounted by all investigators is to show that the effects of LPS in their assay system can be abolished by including polymyxin B (PmB) in cultures, whereas the effects of hsp are unaffected by PmB; conversely LPS is heat

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Table 1. Responses induced by heat shock protein families

Heat shock protein family	Target cell	Effect demonstrated
Hsp60	Monocyte/macrophages	Cytokine production: IL-1, TNFα, IL-6, IL-12 [24], IL-15, Nitric oxide production [13]
	Endothelial cells	Increased expression of CD62e, CD106, CD54 [14, 25]
	Vascular smooth muscle	IL-6 [14]
	Bone calvaria	Resorption – calcium release [26]
Hsp70	Monocyte/macrophages	Cytokine production: IL-1, TNFα [16]
Hsp90	Dendritic cells	Maturation: increased CD83, CD86
		Cytokine production: IL-12, TNFα, GM-CSF [27, 28]
Hsp27, Hsp10	Bone calvaria	Resorption – calcium release [26]

resistant whereas heating hsp preparations significantly decreases their activity.

However, it is still possible that these controls are not entirely adequate – what if hsp are up to their usual chaperoning tricks and are protecting small amounts of LPS and ensuring delivery to cellular receptors – much as they ensure and optimize delivery of antigenic peptides to antigen presenting cells. Under these circumstances the hsp would shield the LPS from binding to PmB, whilst destroying hsp by heating would remove their ability to transport small amounts of LPS. This is a crucial point which requires attention in the design and interpretation of future experiments. There is some evidence against the idea: Asea and colleagues showed that hsp70 induction of TNFα was blocked by a chelator of intracellular calcium whereas there was no such effect on the actions of LPS [16]. This clearly points to differing intracellular signalling mechanisms, but differences between signalling components utilized by hsp and LPS are conspicuously absent from the rest of the literature.

The same argument with respect to LPS could apply to other bacterial components which might find their way into recombinant preparations and interact with receptors of the innate immune system, particularly the Toll-like receptors (TLR). In addition to LPS which interacts with TLR4, bacterial peptidoglycans and lipoproteins interact with TLR2 [18], bacterial CpG DNA with TLR9 [19], flagellin with TLR5 [20], and viral double-stranded RNA with TLR3 [21]. This still leaves several additional TLR whose ligands are currently unknown but may well be products of bacteria.

There is now good evidence, from experiments in TLR4 deficient mice, that hsp60 requires TLR4 to elicit responses, and the same signalling molecules downstream of TLR4 (MyD88, TRAF6) which are critical in LPS signalling are also involved. It has also been suggested that TLR2 can also mediate responses to human hsp60 [22]; this would imply promiscuous association between hsp60 and two different TLRs – as was originally suggested for LPS before contaminants in LPS were identified which accounted for all of the response mediated through TLR2. Alternatively the observations would be explained if hsp60 were able to deliver LPS (or an alternative bacterial ligand) to TLR4, and a peptidoglycan-like entity to TLR2. Other aspects of the mechanism whereby LPS interacts with cells are worth recalling; the interaction is rather complex involving LPS binding protein which greatly increases the potency of LPS by enhancing delivery and binding to CD14. CD14 is indispensible for the LPS response, but seems to pass LPS on to TLR4 which is the signalling receptor. The TLR4 response in turn is significantly augmented by the molecule MD-2. This is produced by the responding cell and associates with TLR4, augmenting responses even when present at picomolar concentrations [23]. These two amplification mechanisms, LPS binding protein and MD-2, suggest that there might be scope for hsp to act in similar ways rather than as direct ligands of the signalling molecule, in addition to the postulated role of 'chaperoning' bacterial components to TLRs. It is unclear whether MD-2 is required for responses to hsp, and data on inhibition of responses to hsp by antibodies to CD14 are conflicting.

To clarify the direct stimulatory role of hsp several questions need to be answered. What is the nature of the interaction between hsp and TLRs, or other receptors such as CD91? Are these interactions specific and can they be mimicked by hsp-derived peptides? Ideally cocrystallization of hsp and receptor and direct measurement of affinity by surface plasmon resonance should be performed. TLR gene knock-out mice may also provide examples where responses to some hsp are maintained whilst those to bacterial components are lost. There is much work to do; delivery of antigenic peptides coupled with direct adjuvant function would represent a major physiological role for hsp in the initiation of immune responses, so we need to be confident of the mechanisms involved.

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